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# (54) Cleaning method and formulation

(57) A method for cleaning a surface carrying proteinaceous material, which comprises applying to the surface one or more enzymes that digest the material, and then wiping the surface.

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# CLEANING METHOD AND FORMULATION

This invention relates to a cleaning method, and in particular to an enzyme formulation.

Cleaning materials used at present rely on very strong solvents to remove dirt present in surfaces and often still require a low of physical exertion to produce the required results. These cleaners, containing high concentrations of solvents and other harmful chemicals such as ammonia, are harmful to the environment, create a dangerous health and safety situation (they are often inflammable as well as toxic), and can damage surfaces they are applied to, especially paintwork.

Dirt on surfaces often contains a biological component which often acts like a "glue" which binds together soil particles and makes the dirt harder to remove. A particular example is of squashed flies or other insects that accumulate on car windscreens and paintwork, which smear over the surface and bind dust and debris to it.

The present invention is based on the realisation that, in order to solve the problems described above, biological catalysts can be used to speed up the reactions that naturally occur between the chemicals present in the environment or supplied, and the matter on the surface to be cleaned, thereby breaking down, or "digesting" the matter. This will enable the material to be removed from the surface more easily.

A formulation for use in the invention will usually contain a number of enzymes; the exact types used and their relative concentrations can be modified to tailor the formulation to the exact purpose to which it is to be put. This can be related to the "average" composition of the biological material on the surfaces. In the case of the windscreens or paintwork covered in squashed flies and road debris, enzymes such as chitinases, proteases and cellulases prove effective in "cutting up" the "glue" and making the surface easy to clean.

The inclusion of proteas s allows the enzymes (which are proteins themselves) to be degraded and so to reduce the activity of the enzymes with time. The formulation is thus "environmentally friendly". It has been found that this action is relatively slow, at least insofar as it is desirable to ensure that the non-protease activity is not compromised in use of the formulation.

The formulation will usually be used in aqueous or other liquid form. No surfactant is necessary. For storage, the components may be freeze-dried.

A liquid formulation can be delivered to the surface to be cleaned by means of a pump action spray. Alternatively, it may be used in the car wash bottle, enabling easy application to the windscreen and headlights.

The enzymes may be free in solution, or they may be on some support, such as kaolin, kieselguhr or diatomite earth. This has the added bonus of having a mild abrasive effect, allowing even more effective cleaning of the surfaces.

The solution of the enzymes can be either aqueous, or contain more powerful solvents to enable an additional "dry cleaning" effect on the surface, by dissolving away the materials present.

Proteases suitable for use in the invention are widely-available. Sources include Sigma, SAF, Boehringer and Biocatalysts Ltd. They may be obtained from various organisms, including mammals, bacteria and fungi.

Insect material (from flies, gnats, moths, butterflies, etc) is composed of proteinaceous material, liquid, cellulose, pectin, hemicellulose, xylans, chitins and a variety of carbohydrates. Enzymes for use in the invention may be chosen accordingly, although not all substrates necessarily have to be digested, in order to utilise the invention.

The following Examples illustrate the invention.

Example 1

General degradation of insect material was assessed using a crude digestion assay system comprising of insect material (whole or crushed, wet and dried samples) of 150-750 mg wet weight in 0.5 ml buffer (phosphate or Tris 50-300 mM at pH 6.5-7.5, preferably 7.0), all incubated at 25-37°C, preferably 30°C. To the substrate mixture, 0.2 ml of biocatalyst suspension was added: suspensions of commercially-available enzymes were used neat (when in liquid) or resuspended freeze-dried samples in phosphate or Tris buffers as above. Reactions were incubated for 20 minutes but not stirred or shaken. The production of general amino-acid content was measured against a suitable background control, using TLC, MPLC, Folin & Ciocalteu's Reagent, Brilliant Blue G or other standard amino-acid and peptide assays known to those skilled in the art. Proteases from Bacillus, Aspergillus, Rhizopus and Streptomyces were particularly effective at releasing large quantities of amino-acids within 5-10 minutes of incubation.

Experiments using different combinations of protease mixtures showed an "additive" effect, particularly with combinations of bacterial and fungal enzymes. This indicated different cleavage mechanisms and kinetics of the individual component enzymes of each formulation; the resultant effect was concomitant release of substantially more amino-acids than with either component enzyme alone, even after prolonged incubation at 37°C.

These results indicated that commercial protease formulations (crude preps) were individually and collectively useful at degrading some of the substructure of insect material, resulting in a loosening of the cellulose-based fabric and exoskeleton and enabling greater accessibility for further breakdown by other enzyme preparations.

Example 2

Using the same experimental procedures as in Example 1 and assay techniques familiar to those skilled in the art, insect substrates were exposed to a wide variety of amylases, glucanases, xylanases, hemicellulases, cellulases, pectinases and chitinases. The corresponding release of sugar products was followed using standard sugar assay techniques. All enzyme preparations tested were able to release considerable quantities of saccharides, disaccharides and polysaccharides from ground insect material.

Example 3

Combinations of cellulases from Aspergillus and Trichosporon, proteases from Streptomyces, Aspergillus, Rhizopus, amylases from Bacillus and Aspergillus, and lipases from Candida, Mucor and Rhizopus, were tested in a variety of different concentrations upon insect material (100 mg in 3 ml 50 mM buffer, pH 7.0, at 25°C). Release of sugars, amino-acids, fatty acids, etc was monitored using HPLC and shown to occur simultaneously over an initial period (3-7 minutes), but decreased after 15 minutes, probably due to cannibalism of the other lytic enzymes by the powerful protease content.

These experiments indicated that a variety of enzyme activities could perform to the same degree in the presence of each other's activities with release of water-soluble monounits and concomitant collapse of the insect structure.

Example 4

Bottles of enzyme formulations were prepared using 5% total enzyme concentrations with varying mixtures of proteases, lipases, cellulases and amylases from 0-100% of the enzyme content of each formulation. In addition, abrasive agents such as vermiculite, kieselguhr and Fuller's earth were added in finely ground particulate form and ethanol (0.5-2%) was mixed into the final formulation.

Windscreens with dried insect debris were prepared, throughout June and July, by driving through country lanes,

and not using the windscreen wash prior to the xperiments. 500 ml bottles of each formulation were prepared, shaken vigorously before use and sprayed (using a standard pump and nozzle bottle) over one half of the windscreen. The other half was protected by newspaper to prevent carry-over. Each treated area was left for 10 minutes before applying the standard windscreen wash and wipers (water and detergent) with 10 swipes of the wipers. All of the formulations had some effect, better than the use of water or buffer alone.

The most marked removal of insect debris was achieved using formulations containing 40-60% protease, 10-30% lipase, 20-40% cellulase and 10-20% amylase. The optimum formulation appeared to be 40% protease (fungal and bacterial mix), 10% lipase, 35% cellulase and 15% amylase.

The presence of vermiculite or Fuller's earth particles improved the clearance of debris from the windscreen, as did ethanol at up to 20%.

CLAIMS

1. A method for cleaning a surface carrying proteinaceous material, which comprises applying to the surface one or more enzymes that digest the material, and then wiping the surface.

2. A method according to claim 1, wherein the surface is an automobile windscreen.

3. A method according to claim 1 or claim 2, wherein the one or more enzymes are applied in a liquid film over the surface, by operation of an applicator containing the liquid.

4. A method according to claims 2 and 3, wherein the one or more enzymes are applied as a component of the windscreen wash liquid.

5. A method according to any preceding claim, wherein the one or more enzymes are selected from chitinases, cellulases and proteases.

6. A method according to any preceding claim, wherein a clay-like particulate material is also applied.

7. A method according to any preceding claim, wherein the enzymes are applied in an aqueous system comprising ethanol.

8. A method according to claim 1, substantially as exemplified herein.

9. A formulation comprising the enzymes defined in claim 5.

10. A formulation according to claim 9, additionally comprising a clay-like particulate material.

11. A formulation comprising protease and a clay-like particulate material.

12. A formulation according to claim 11, additionally comprising chitinase and/or cellulase.

13. A formulation according to any of claims 9 to 12, in an aqueous medium comprising ethanol.

14. A formulation according to any of claims 9 to 13, comprising bacterial and fungal proteases.

| Patents Act 1977<br>Examiner's report to the Comptroller under Section 17<br>(The Search report) | Application number<br>GB 9423453.1 |
|---|---|
| **Relevant Technical Fields**<br><br>(i) UK Cl (Ed.) C5D DGA<br><br>(ii) Int Cl (Ed.) | Search Examiner<br>M ELLIOTT<br><br>Date of completion of Search<br>10 FEBRUARY 1995 |
| **Databases** (see below)<br>(i) UK Patent Office collections of GB, EP, WO and US patent specifications.<br><br>(ii) | Documents considered relevant following a search in respect of Claims :-<br>1-14 |

**Categories of documents**

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|---|---|---|---|
| X | EP 0181651 A2 | (UNILEV) whole document | 1 at least |
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